

Quick and Easy Characterization of Microplastics in Surface Water and Treated Effluent

Analyzing microplastic contamination using an Agilent 8700 LDIR Chemical Imaging System



Authors

Danence Lee Ph.D
PUB, Singapore's National Water
Agency

Siji Joseph, Chee Ping Wong,
Darren Robey, Chee Sian Gan
Agilent Technologies, Inc.

Introduction

Contamination of microplastics in many water sources is a serious threat to the environment (1, 2). As the number of particles in water may span many orders of magnitude, the potential harmful impact of exposure to microplastics is a hot topic in the scientific community. It is often challenging to measure the size and number of microplastic particles in water using traditional manual spectroscopy such as Fourier Transform Infrared (FTIR) or visual approaches. Extensive literature references are available on sample cleanup procedures required to prepare microplastics for analysis. However, the procedures are typically time-consuming and include hazardous chemicals, with the added difficulty of handling small sample sizes. While FTIR is the traditional choice for microplastics, data collection is extremely time-consuming, often taking days. The large coherent source of an FTIR is quite inefficient at focusing on small microplastic particles. In this study, we evaluate a simpler approach to isolating microplastics from various water matrices and identifying them using an Agilent 8700 Laser Direct Infrared (LDIR) Chemical Imaging System.

Experimental

Reagents and chemicals

Spectroscopy-grade ethanol, sodium dodecyl sulfate and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). A polystyrene (PS) 80 μm microsphere sized standard was purchased from Thermo Scientific (Fremont, CA, USA). The de-ionized (DI) water used for the spike recovery analysis was collected from a Milli Q water purification system (Millipore, USA).

Water sampling and laboratory preparation

A total of 10 L of bulk surface water from a water catchment and treated effluent water from a waste water facility, both in Singapore was collected using a jerrycan. 1 L of water was then subsampled for microplastic analysis. All glassware was treated with 0.3 g/L sodium dodecyl sulfate solution in an ultrasonic bath and rinsed with ultrapure (18 M Ω) water before use.

Particle collection workflow

The particle collection procedure is outlined in Figure 1.

The vacuum filtration equipment was bought from Johnson Scientific (Johor, Malaysia). The water samples were vacuum filtered using an Agilent 10 μm , 19 mm semi prep filter disc (part number: 5022-2166, Figure 2), with suitable hardware assembly (p/n: 5022-2165). The filter disc is an easily removable and reusable stainless-steel mesh screen. To trap the particles, the sample was directed to the filter disc assembly using PTFE solvent tubing (p/n: 5062-2483) connected with frit adapters (p/n: 5062-8517). Each filtration event took approximately 7 to 10 min, depending on the complexity of the sample type. After filtering complex samples like surface water and treated effluent, the filter disc was flushed with 50 mL DI water to rinse the trapped particles. To avoid cross-contamination between each filtration event, the filter discs were forward- and backward-flushed with DI water.



Figure 2. The pre-prep filter disk used to collect the microplastic particles

The particles that were collected on the steel filter disc were quantitatively transferred into 2 mL ethanol using ultrasonic treatment in a beaker. The resulting particle-ethanol suspension was evaporated to dryness by placing the beaker on a hot plate maintained at 70 $^{\circ}\text{C}$ and later resuspended in 200 μL ethanol. The final resuspended ethanol layer was transferred onto two infrared reflective glass slides (7.5 \times 2.5 cm; MirrIR, Kevley Technologies, Ohio, USA) using pipettes. The pipette tip was manually cut to widen the aperture to accommodate particles up to a maximum size of 1000 μm . To minimize contamination by air, all microplastic particle preparation steps were performed in a laminar airflow fume hood. The final glass plates were stored in closed glass Petri dishes before analysis by LDIR.

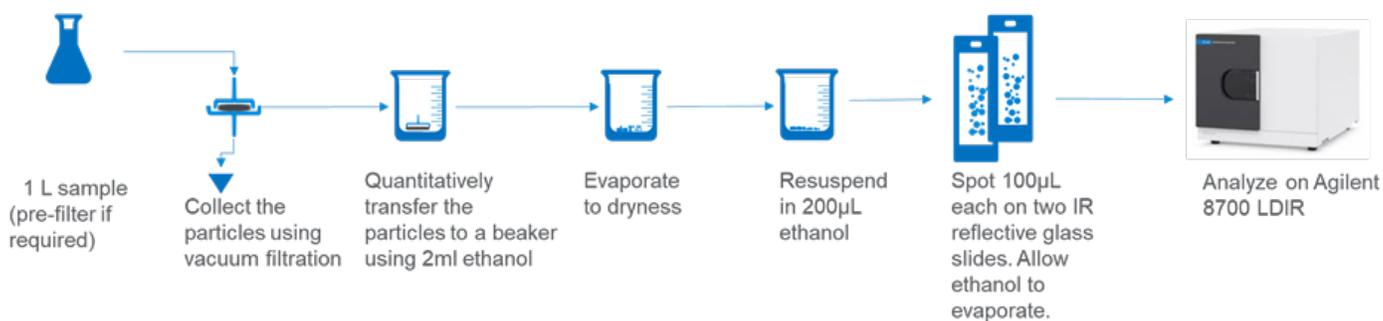


Figure 1. Microplastic particle collection workflow.

Spiked DI water samples as positive controls

A challenge of analyzing a wide range of particle sizes from 20–1000 μm is the loss of particles during the sample preparation procedure. To verify the sample processing efficiency and to quantify any particle loss, a recovery analysis using positive controls was included in the experimental design. Six technical replicates of the positive controls were prepared by spiking about 100 microsphere-shaped 80 μm PS particles in 1 L of DI water. The percentage of 80 μm PS beads retrieved from each positive control was calculated and the average value was expressed as recovery (%). The reproducibility of the procedure was evaluated by calculating the relative standard deviation (RSD) of recovery from the six technical replicates.

Particle collection from water sources

The microplastic particles from the surface and treated effluent water samples were collected using the workflow outlined in Figure 1. Before particle collection, the surface and treated effluent water samples were pre-sieved with a 1000 μm metal sieve mesh to remove any larger particles. The sample preparation workflow was performed in duplicate using each water source and averaged information of particles was used for comparison purposes.

Similar to the positive control samples, a known number of 80 μm PS beads were spiked into 1 L each of surface and treated effluent water, and the recovery was estimated. The spiking was performed before the pre-sieving step to accommodate any loss while filtering through a 1000 μm metal sieve.

Instrumentation

An Agilent 8700 LDIR Chemical Imaging System controlled using Agilent Clarity software (version: 1.2.19) was used in this study. The infrared reflective glass slides plated with collected particles from each sample were analyzed by the 8700 LDIR simply by selecting the preloaded microplastics analysis method from the Clarity software. The 8700 LDIR system uses a quantum cascade laser (QCL) source, which provides fast IR scanning data acquisition of the selected area to locate all the particles within the selected dimension range. The system collects high-quality spectral imaging data of each particle and the software automatically performs a library search to confirm the particle's identity. The method setup parameters used for data acquisition are shown in Figure 3.

The Clarity software identifies each particle in 10 seconds, enabling the fast analysis of microplastics in water samples. The software automatically generates a report with the total number of particles identified, the size measurement of each particle with width, height, diameter, perimeter, solidity, and reference library matching quality.

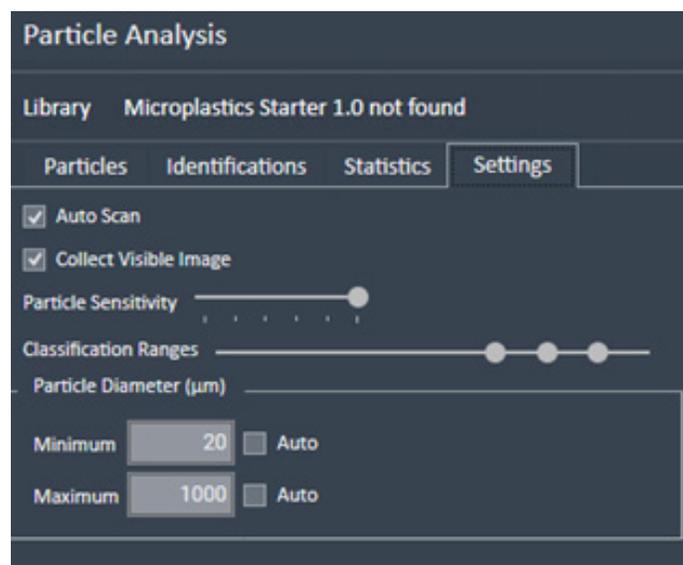


Figure 3. Microplastic analysis method parameters.

Analysts may also consider the following tips:

- The right side of the Kevley infrared reflective glass slides was blackened using a permanent marker to limit the light entering from the side directions and interfering with the LDIR sample profiling step.
- The final resuspended ethanol can be partially plated on Kevley infrared reflective glass slides to control the particle intensity. However, the calculation to count recovered particles and filtration efficiency must be adjusted to account for the plated solvent subset volume.
- The interference from inorganic salts (originating from calcium and magnesium salts) in surface and treated effluent water samples can be minimized by sonication with a 250 g/L EDTA solution. Wait 15 min before filtration.
- The confidence and quality of microplastic identification from complex water samples can be further improved by organic and inorganic digestion steps as described elsewhere (2).

Results and discussion

The Agilent 8700 LDIR chemical imaging system is chemically specific and nondestructive. The LDIR system identified microplastics of different shapes and sizes in all studied water matrices and the Clarity software automatically generated statistical data for fast compositional assessment of the particles. Any identified particles with a library Hit Quality Index (HQI) over 0.8 were considered for further matrix-based statistical analysis.

Sample preparation reliability

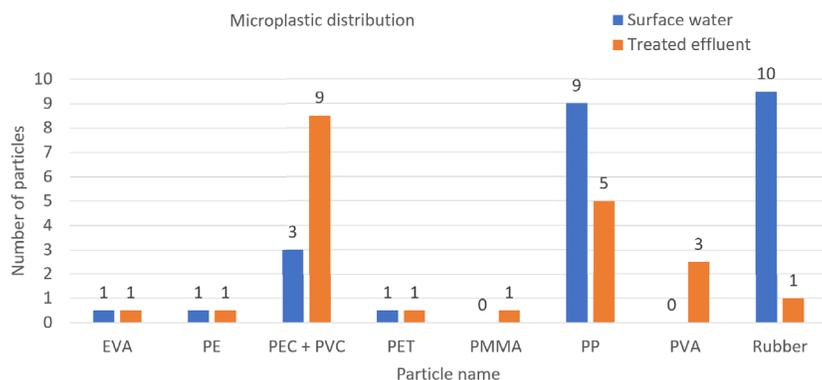
To assess the reliability of the sample preparation procedure, recovery and repeatability of measurement of 80 µm PS beads were calculated following the analysis of six technical replicates of the positive control samples. The observed average recovery was 73% with repeatability of <10% RSD. The good recovery rate and % RSD values confirmed the efficiency of the particle extraction method.

Microplastics from real-life water samples

The 8700 LDIR Chemical Imaging System can identify particles even in the presence of organic or inorganic interferences that arise from the matrix. The QCL infrared source technology, detector, and fast-scanning optics allow the 8700 to obtain an IR spectrum of each microparticle, enabling rapid identification of particles within seconds.

In the surface water sample, 25 microplastic particles with a blend of six known components were measured in the 1 L sample. Polyethylene chlorinated (PEC), polypropylene (PP), and rubber were the most prevalent particles, contributing to >90% of particles. Low levels of ethylene vinyl acetate (EVA), polyethylene (PE), and polyethylene terephthalate (PET) were also detected in surface water. 22 microplastic particles were observed in 1 L of the treated effluent water. PEC, polyvinylchloride (PVC), PP, and polyvinyl alcohol (PVA) were the most predominant microplastic particles. The remaining particles were identified as EVA, PE, PET, polymethylmethacrylate (PMMA), and rubber. A summary of the particles observed in the surface and effluent waters is shown in Figure 4.

The Clarity software can be used to zoom in and examine individual particles when required. Zoomed-in images of rubber (from surface water) and PVC (from treated effluent water) particles with an HQI of >0.8 are shown in Figure 5 as examples.



Abbreviation	Particle	Particle size range (µm)	
		Surface water	Treated effluent
EVA	Ethylene Vinyl Acetate	29	49
PE	Polyethylene (PE)	43	13
PEC + PVC	Polyethylene Chlorinated + Polyvinylchloride	26 - 36	31 - 286
PET	Polyethylene Terephthalate (PET)	80	36
PMMA	Polymethylmethacrylate (PMMA)	-	28
PP	Polypropylene (PP)	30 - 549	63 - 110
PVA	Polyvinyl alcohol	-	42 - 65
Rubber	Rubber	32 - 128	38

Figure 4. Particle summary from the surface and treated effluent water samples.

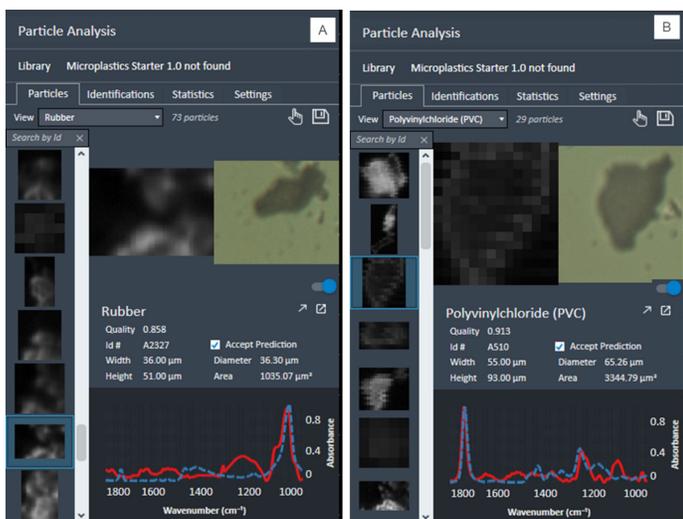


Figure 5. Identification results of rubber from surface water (A) and a PVC particle from treated effluent water (B).

Spike recovery results

The recovery analysis of 80 μm PS beads spiked in surface and treated effluent water samples were also >73%. The robust LDIR spectral quality and library match results enabled confident identification of PS beads even in complex matrices like surface and treated effluent, which often have high matrix backgrounds.

Figure 6 shows the identification results of spiked polystyrene particles from DI, surface, and treated effluent waters (hit quality index >0.895). The increased matrix background from the surface and treated effluent water samples is evident in the high-resolution camera images when compared to DI water (Figure 6).

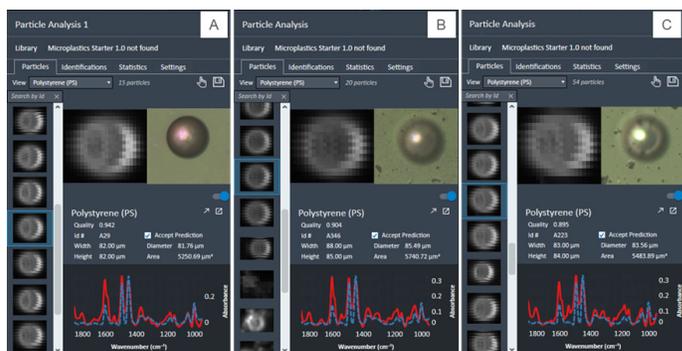


Figure 6. Recovery of PS beads from various water sources. A: DI water, B: surface water, C: treated effluent water.

Automatic statistics on the particle size distribution

The reports feature of Clarity software was used to create statistical data of microplastic particles based on various size ranges. Figure 7 is a summary of particle size range distribution plotted against the percentage of particles observed per sample. Within the size range of 1000 μm being studied, most of the particles in surface and treated effluent water samples ranged between 20-200 μm , as shown in Fig 7 below.

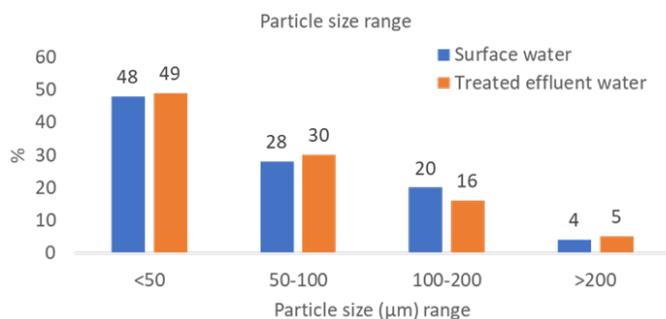


Figure 7. Particle size (μm) distribution in surface and treated effluent water samples.

Conclusion

The study showed the effectiveness of the Agilent 8700 LDIR Chemical Imaging System for the characterization of microplastic particles in environmental waters following a quick and simple sample preparation procedure.

A vacuum filtration using an Agilent 10 µm filter disc was used to collect particles from the water samples. The procedure only took a few minutes to complete, improving the productivity compared to more conventional day-long processes of FTIR. Good particle isolation efficiency (73% recovery) and reproducibility (<10% RSD) of the procedure were verified using DI water samples spiked with known numbers of 80 µm polystyrene beads.

Many of the method and instrument settings were automatically loaded from the Clarity software using a preset microplastics analysis method, greatly simplifying operation even for nonexpert analysts. The focused laser source in the 8700 LDIR enabled fast data collection and reliable characterization of particles as small as to 10 µm size with minimal instrument interaction. More than 20 microparticles per litre (mainly PEC+PVE, PP, rubber) were found in the surface water and treated effluent, with most particles ranging in size from 20 to 200 µm.

The particles were identified automatically by crossmatching with the reference library. Also, the ability to zoom in on the individual particles helped to further investigate the data when required, without changing any optics of the 8700 LDIR.

References

1. N.P. Ivleva *et al*, Microplastic in Aquatic Ecosystems. *Angewandte Chemie Int Ed*, **2017**, 56(7): 1720-1739.
2. Philip J. Anderson *et al*. Microplastic contamination in Lake Winnipeg, Canada, *Enviro Poll.* 225, **2017**, 223e231.

www.agilent.com/chem/8700-ldir

DE44447.0223842593

This information is subject to change without notice.

© Agilent Technologies, Inc. 2021
Printed in the USA, September 30, 2021
5994-3932EN